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FILE 'SCISEARCH' ENTERED AT 15:52:29 ON 28 DEC 2007

FILE 'CAPLUS' ENTERED AT 15:52:29 ON 28 DEC 2007

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=> s single domain antibod?
L1 636 SINGLE DOMAIN ANTIBOD?

=> s l1 and EGFR
L2 10 L1 AND EGFR

=> dup remove l2
PROCESSING COMPLETED FOR L2
L3 2 DUP REMOVE L2 (8 DUPLICATES REMOVED)

=> d l3 1-2 cbib abs

L3 ANSWER 1 OF 2 MEDLINE on STN DUPLICATE 1
2006718898. PubMed ID: 16848761. Studies of thermostability in Camelus bactrianus (Bactrian camel) **single-domain antibody** specific for the mutant epidermal-growth-factor receptor expressed by Pichia. Omidfar Kobra; Rasaee Mohhamad Javad; Kashanian Soheila; Paknejad Malieheh; Bathaie Zahra. (Endocrinology and Metabolism Research Centre, Tehran University of Medical Sciences, Tehran, Iran.) Biotechnology and applied biochemistry, (2007 Jan) Vol. 46, No. Pt 1, pp. 41-9. Journal code: 8609465. E-ISSN: 1470-8744. Pub. country: England: United Kingdom. Language: English.

AB Camelids have a unique immune system capable of producing heavy-chain antibodies lacking the light chains and CH1 (constant heavy-chain domain 1). It has been shown that, in contrast with conventional antibody fragments, the variable domains of these heavy-chain antibodies are functional at or after exposure to high temperatures. In the present study, the VHH (variable domain of heavy-chain antibody) camel antibody was subcloned into vector Ppiczc and expressed in Pichia pastoris. ORB1-83 VHH antibody recognizes the external domain of the mutant **EGFR** [EGF (epidermal growth factor) receptor], **EGFR** VIII. This tumour-specific antigen is ligand-independent, contains a

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 NEWS  3  AUG 06   FSTA enhanced with new thesaurus edition
 NEWS  4  AUG 13   CA/CAplus enhanced with additional kind codes for granted
                   patents
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                   spectral property data
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                   patents
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                   Zentralblatt
 NEWS 16  OCT 19   BEILSTEIN updated with new compounds
 NEWS 17  NOV 15   Derwent Indian patent publication number format enhanced
 NEWS 18  NOV 19   WPIX enhanced with XML display format
 NEWS 19  NOV 30   ICSD reloaded with enhancements
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 NEWS 22  DEC 17   USPATOLD added to additional database clusters
 NEWS 23  DEC 17   IMSDRUGCONF removed from database clusters and STN
 NEWS 24  DEC 17   DGENE now includes more than 10 million sequences
 NEWS 25  DEC 17   TOXCENTER enhanced with 2008 MeSH vocabulary in
                   MEDLINE segment
 NEWS 26  DEC 17   MEDLINE and LMEDLINE updated with 2008 MeSH vocabulary
 NEWS 27  DEC 17   CA/CAplus enhanced with new custom IPC display formats
 NEWS 28  DEC 17   STN Viewer enhanced with full-text patent content
                   from USPATOLD

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constitutively active tyrosine kinase domain and has been shown to be present in a number of human malignancies. We report here that, although expression from P. pastoris resulted in a significantly increased level of expression of the anti-**EGFR** VIII VHH antibodies compared with Escherichia coli [Omidfar, Rasaee, Modjtahedi, Forouzandeh, Taghikhani, Bakhtiari, Paknejad and Kashanian (2004) Tumor Biol. 25, 179-187; Omidfar, Rasaee, Modjtahedi, Forouzandeh, Taghikhani and Golmakany (2004) Tumor Biol. 25, 296-305], this antibody selectively bound to the **EGFR** VIII peptide and reacted specifically with the immunoaffinity-purified antigen from non-small-cell lung cancer. Furthermore, thermal denaturation stability and CD spectra analysis of the Camelus bactrianus (Bactrian camel) VHH and heavy-chain antibodies at different temperature proved reversibility and binding activity after heat denaturation. Our results indicate that the P. pastoris expression system may be useful for the expression of camel **single domain antibody** and the ability of the expressed protein to reversibly melt without aggregation, allowing it to regain binding activity after heat denaturation.

L3 ANSWER 2 OF 2 MEDLINE on STN DUPLICATE 2

2005002331. PubMed ID: 15627895. Production of a novel camel **single-domain antibody** specific for the type III mutant **EGFR.** Omidfar K; Rasaee M J; Modjtahedi H; Forouzandeh M; Taghikhani M; Golmakani N. (Department of Biochemistry, School of Medical Sciences, Tarbiat Modarres University, Tehran, Iran.) Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine, (2004 Sep-Dec) Vol. 25, No. 5-6, pp. 296-305. Journal code: 8409922. ISSN: 1010-4283. Pub. country: Switzerland. Language: English.

AB Camelids have a unique immune system capable of producing single-domain heavy-chain antibodies. The antigen-specific domain of these heavy-chain IgGs (VHH) are the smallest binding units produced by the immune system. In this study, we report the isolation and characterization of several binders against the epidermal growth factor receptor (**EGFR**) vIII retrieved from immune library of camels (Camelus bactrianus and Camelus dromedarius). The EGFRvIII is a ligand-independent, constitutively active, mutated form of the wild-type **EGFR.** The expression of EGFRvIII has been demonstrated in a wide range of human malignancies, including gliomas, and breast, prostate, ovarian and lung cancer. Camels were immunized with a synthetic peptide corresponding to a mutated sequence and tissue homogenates. **Single-domain antibodies** (VHH) were directly selected by panning a phage display library on successively decreasing amounts of synthetic peptide immobilized on magnetic beads. The anti-EGFRvIII camel **single-domain antibodies** selectively bound to the EGFRvIII peptide and reacted specifically with the immunoaffinity-purified antigen from a non-small cell lung cancer patient. These antibodies with affinities in the nanomolar range recognized the EGFRvIII peptide and affinity-purified mutated receptor. We concluded that using the phage display technique, antigen-specific VHH antibody fragments are readily accessible from the camelids. These antibodies may be good candidates for tumor-diagnostic and therapeutic applications.


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=> s VHH antibody
L4          84 VHH ANTIBODY

=> s l4 and EGFR
L5          11 L4 AND EGFR

=> dup remove l5
PROCESSING COMPLETED FOR L5
L6           3 DUP REMOVE L5 (8 DUPLICATES REMOVED)
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=> d l6 1-3 cbib abs

L6 ANSWER 1 OF 3 MEDLINE on STN DUPLICATE 1
2006718898. PubMed ID: 16848761. Studies of thermostability in Camelus bactrianus (Bactrian camel) single-domain antibody specific for the mutant epidermal-growth-factor receptor expressed by Pichia. Omidfar Kobra; Rasaee Mohhamad Javad; Kashanian Soheila; Paknejad Malieheh; Bathaie Zahra. (Endocrinology and Metabolism Research Centre, Tehran University of Medical Sciences, Tehran, Iran.) Biotechnology and applied biochemistry, (2007 Jan) Vol. 46, No. Pt 1, pp. 41-9. Journal code: 8609465. E-ISSN: 1470-8744. Pub. country: England: United Kingdom. Language: English.

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L6 ANSWER 2 OF 3 MEDLINE on STN DUPLICATE 2
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antigen from a non-small cell lung cancer patient. These antibodies with affinities in the nanomolar range recognized the EGFRvIII peptide and affinity-purified mutated receptor. We concluded that using the phage display technique, antigen-specific **VHH antibody** fragments are readily accessible from the camelids. These antibodies may be good candidates for tumor-diagnostic and therapeutic applications. Copyright 2004 S. Karger AG, Basel.

L6 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2007 ACS on STN
2005:520389 Document No. 143:265130 Production of antibody against **EGFR** V III and the other EGF receptor family and molecular cloning of heavy chain antibody repertoire of Camelus bactrianus and Camelus dromedarius. Omidfar, K.; Rasaee, M. J.; Forouzandeh Moghadam, M.; Taghikhani, M.; Modjtahedi, H.; Sadroddiny, E. (Biochemistry Department, School of Medical Sciences, Tarbiat Modarres University, Tehran, Iran). Majmoa-i Maghalat-i Sevomin Hemayesh Maliy Biotechnology Jomhoriy-i Islame-i Iran, Mashhad, Islamic Republic of Iran, Sept. 9-11, 2003, Volume 3, 42-45. Danishgah-i Ferdowsi Mashhad: Mashhad, Iran. ISBN: 964-386-023-X (English) 2003. CODEN: 69GXPF.
AB The epidermal growth factor receptor (**EGFR**) is a membrane glycoprotein that possesses intrinsic protein tyrosine kinase activity and mediates proliferation and differentiation of cells when activated by its ligand EGF or transforming growth factor α (TGF α). Many cancerous cell have been shown to express mutant form of this receptor. The most common receptor mutant, **EGFR** V III, is known to be present in glioblastomas, breast and ovarian cancers, non-small cell lung carcinomas and prostate cancers. This mutated receptor has previously been described and is formed by a 267 amino acid in-frame deletion and an insertion of a glycine in the fusion of the extra cellular domain. Camelidae are known to produce antibodies devoid of light chains and CH1 domains. Antigen-specific fragments. of these heavy-chain IgGs (VHH) are of great interest in biotechnol. applications. In this study we report the novel polyclonal camel antibodies directed to the mutation site of **EGFR** V III. These antibodies were generated by immunization of camelids with a synthetic peptide corresponding to the mutated sequence of the receptor, tissue homogenize of several patient with human glioblastoma, medulloblastoma and aggressive breast carcinoma as well as EGF receptor expression cell lines. The elicited antibody reacted specifically with the fusion peptide in ELISA. The anti-fusion junction peptide antibody selectively bound to the mutated receptor as compared to the intact epidermal growth factor receptor as assessed by immunocytochem. and Western blot anal. In addition to enable the specific and efficient isolation of VHH genes from peripheral blood B-cells, the long and short-hinge specific primers were used in the construction of camelids VHH libraries. We concluded that, using the technique described, antigen-specific **VHH antibody** fragments are readily accessible from the camelids as well as these antibodies are ideal candidates for tumor diagnostic and therapeutic applications.

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=> s antibod?
L7     3060346 ANTIBOD?

=> s l7 and EGFR
L8        9505 L7 AND EGFR

=> s l8 adn cameliadae
MISSING OPERATOR L8 ADN
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=> s l8 and cameliadae
L9           0 L8 AND CAMELIADAE

=> s l8 and VHH
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L10 16 L8 AND VHH

=> dup remove l10
PROCESSING COMPLETED FOR L10
L11 7 DUP REMOVE L10 (9 DUPLICATES REMOVED)

=> d l11 1-7 cbib abs

L11 ANSWER 1 OF 7 MEDLINE on STN DUPLICATE 1
2006718898. PubMed ID: 16848761. Studies of thermostability in Camelus bactrianus (Bactrian camel) single-domain **antibody** specific for the mutant epidermal-growth-factor receptor expressed by Pichia. Omidfar Kobra; Rasaee Mohhamad Javad; Kashanian Soheila; Paknejad Malieheh; Bathaie Zahra. (Endocrinology and Metabolism Research Centre, Tehran University of Medical Sciences, Tehran, Iran.) Biotechnology and applied biochemistry, (2007 Jan) Vol. 46, No. Pt 1, pp. 41-9. Journal code: 8609465. E-ISSN: 1470-8744. Pub. country: England: United Kingdom. Language: English.

AB Camelids have a unique immune system capable of producing heavy-chain **antibodies** lacking the light chains and CH1 (constant heavy-chain domain 1). It has been shown that, in contrast with conventional **antibody** fragments, the variable domains of these heavy-chain **antibodies** are functional at or after exposure to high temperatures. In the present study, the **VHH** (variable domain of heavy-chain **antibody**) camel **antibody** was subcloned into vector Ppiczc and expressed in Pichia pastoris. ORB1-83 **VHH** **antibody** recognizes the external domain of the mutant **EGFR** [EGF (epidermal growth factor) receptor], **EGFR** VIII. This tumour-specific antigen is ligand-independent, contains a constitutively active tyrosine kinase domain and has been shown to be present in a number of human malignancies. We report here that, although expression from P. pastoris resulted in a significantly increased level of expression of the anti-**EGFR** VIII **VHH** **antibodies** compared with Escherichia coli [Omidfar, Rasaee, Modjtahedi, Forouzandeh, Taghikhani, Bakhtiari, Paknejad and Kashanian (2004) Tumor Biol. 25, 179-187; Omidfar, Rasaee, Modjtahedi, Forouzandeh, Taghikhani and Golmakany (2004) Tumor Biol. 25, 296-305], this **antibody** selectively bound to the **EGFR** VIII peptide and reacted specifically with the immunoaffinity-purified antigen from non-small-cell lung cancer. Furthermore, thermal denaturation stability and CD spectra analysis of the Camelus bactrianus (Bactrian camel) **VHH** and heavy-chain **antibodies** at different temperature proved reversibility and binding activity after heat denaturation. Our results indicate that the P. pastoris expression system may be useful for the expression of camel single domain **antibody** and the ability of the expressed protein to reversibly melt without aggregation, allowing it to regain binding activity after heat denaturation.

L11 ANSWER 2 OF 7 CAPLUS COPYRIGHT 2007 ACS on STN
2006:763683 Document No. 145:187073 Methods for identifying, selecting, cloning and generating small intact antigen-binding nanobodies comprising heavy chain variable domain sequences. Hermans, Guy; De Haard, Johannes Joseph Wilhelmus (Ablynx N.V., Belg.). PCT Int. Appl. WO 2006079372 A1 20060803, 174pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IS, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2005-EP11819 20051104. PRIORITY: US 2005-648922P 20050131; US 2005-663622P 20050318.

AB The present invention relates to a method for generating or cloning a nucleic acid or nucleotide sequence that encodes a heavy chain

antibody or an antigen-binding fragment thereof, wherein said heavy chain **antibody** or antigen-binding fragment is directed against a specific antigen, said method comprising the steps of providing a sample or population of cells from a Camelid immunized with said antigen, isolating from said sample or population said at least one cell that expresses or is capable of expressing a heavy chain **antibody** directed against said antigen, and obtaining from said at least one cell a nucleic acid or nucleotide sequence that encodes a heavy chain **antibody** directed against antigen or that encodes an antigen-binding fragment thereof directed against said antigen. The heavy chain variable domain is e.g. a camelid **VHH** fragment specific to a desired antigen. In examples, llama anti-human **EGFR**, anti-human C1q, anti-collagen, anti-human CD28, anti-human integrin αvβ5 and anti-human TNF monospecific or bispecific nanobodies were prepared

L11 ANSWER 3 OF 7 CAPLUS COPYRIGHT 2007 ACS on STN
2006:977711 Document No. 145:334114 Method for generating variable domain sequences of heavy chain **antibodies.** Hermans, Guy; De Haard, Johannes Joseph Wilhelmus (Ablynx N.V., Belg.). U.S. Pat. Appl. Publ. US 2006211088 A1 20060921, 100pp., Cont.-in-part of U.S. Ser. No. 343,972. (English). CODEN: USXXCO. APPLICATION: US 2006-375679 20060313. PRIORITY: US 2005-648922P 20050131; US 2005-663622P 20050318; WO 2005-EP11819 20051104; US 2006-343972 20060131.

AB The authors disclose a method for the generation, selection, and cloning of heavy chain (camelid) **antibodies.** The method comprises elicitation and isolation of a population of cells from a camelid immunized with a target antigen and obtaining from the cells a nucleic acid or nucleotide sequence that encodes a heavy chain **antibody** directed against the target antigen. In one example, the authors elicit and isolate llama B-cells with specificity for human EGF receptor and, using a homologous recombination technique, clone the **VHH** domain of anti-**EGFR antibodies.**

L11 ANSWER 4 OF 7 CAPLUS COPYRIGHT 2007 ACS on STN
2005:429443 Document No. 142:480780 Camelid heavy chain **antibodies** directed against epidermal growth factor receptor. Laeremans, Toon (Ablynx N. V., Belg.). PCT Int. Appl. WO 2005044858 A1 20050519, 78 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-BE189 20031107.

AB The author discloses the preparation, selection, and characterization of llama **antibodies** directed to human epidermal growth factor receptor.

L11 ANSWER 5 OF 7 MEDLINE on STN DUPLICATE 2
2005002331. PubMed ID: 15627895. Production of a novel camel single-domain **antibody** specific for the type III mutant **EGFR.** Omidfar K; Rasaee M J; Modjtahedi H; Forouzandeh M; Taghikhani M; Golmakani N. (Department of Biochemistry, School of Medical Sciences, Tarbiat Modarres University, Tehran, Iran.) Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine, (2004 Sep-Dec) Vol. 25, No. 5-6, pp. 296-305. Journal code: 8409922. ISSN: 1010-4283. Pub. country: Switzerland. Language: English.

AB Camelids have a unique immune system capable of producing single-domain heavy-chain **antibodies.** The antigen-specific domain of these heavy-chain IgGs (**VHH**) are the smallest binding units produced by the immune system. In this study, we report the isolation and characterization of several binders against the epidermal growth factor receptor (**EGFR**) vIII retrieved from immune library of camels

(Camelus bactrianus and Camelus dromedarius). The EGFRvIII is a ligand-independent, constitutively active, mutated form of the wild-type **EGFR.** The expression of EGFRvIII has been demonstrated in a wide range of human malignancies, including gliomas, and breast, prostate, ovarian and lung cancer. Camels were immunized with a synthetic peptide corresponding to a mutated sequence and tissue homogenates. Single-domain **antibodies** (**VHH**) were directly selected by panning a phage display library on successively decreasing amounts of synthetic peptide immobilized on magnetic beads. The anti-EGFRvIII camel single-domain **antibodies** selectively bound to the EGFRvIII peptide and reacted specifically with the immunoaffinity-purified antigen from a non-small cell lung cancer patient. These **antibodies** with affinities in the nanomolar range recognized the EGFRvIII peptide and affinity-purified mutated receptor. We concluded that using the phage display technique, antigen-specific **VHH antibody** fragments are readily accessible from the camelids. These **antibodies** may be good candidates for tumor-diagnostic and therapeutic applications.

L11 ANSWER 6 OF 7 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 3

2005:84957 Document No.: PREV200500086004. Production and characterization of a new **antibody** specific for the mutant EGF receptor, EGFRvIII, in Camelus bactrianus. Omidfar, K.; Rasaee, M. J. [Reprint Author]; Modjtahedi, H.; Forouzandeh, M.; Taghikhani, M.; Bakhtiari, A.; Paknejad, M.; Kashanian, S.. Sch Med SciDept Biochem, Tarbiat Modarres Univ, POB 14115-331, Tehran, Iran. rasaee_m@modares.ac.ir. Tumor Biology, (2004) Vol. 25, No. 4, pp. 179-187. print.
ISSN: 1010-4283 (ISSN print). Language: English.

AB EGFRvIII is the type III deletion mutant form of the epidermal growth factor receptor (**EGFR**) with transforming activity. This tumor-specific antigen is ligand independent, contains a constitutively active tyrosine kinase domain and has been shown to be present in a number of human malignancies. In this study, we report the production and characterization of camel **antibodies** that are directed against the external domain of the EGFRvIII. **Antibodies** developed in camels are smaller (i.e. IgG2 and IgG3 subclasses lack light chains) than any other conventional mammalian **antibodies.** This property of camel **antibodies** makes them ideal tools for basic research and other applications such as tumor imaging and cancer therapy. In the present study, camel **antibodies** were generated by immunization of camelids (Camelus bactrianus and Camelus dromedarius) with a synthetic 14-amino acid peptide corresponding to the mutated sequence of the **EGFR**, tissue homogenates of several patients with human glioblastoma, medulloblastoma and aggressive breast carcinoma, as well as **EGFR**-expressing cell lines. Three subclasses of camel IgG (conventional (IgG1, 160 kD) and heavy chain-only **antibodies** (IgG2 and IgG3, 90 kD)) were separated by their different binding properties to protein A and protein G affinity columns. The anti-EGFRvIII peptide **antibodies** from immunized camels were purified further using the EGFRvIII synthetic peptide affinity column. The purified anti-EGFRvIII peptide camel **antibodies** selectively bound to the EGFRvIII peptide and affinity-purified EGFRvIII from malignant tissues and detected a protein band of 140 kD from malignant tissues by Western blot. Affinity analysis showed that the **antibodies** from C. bactrianus and C. dromedarius reacted with peptide and antigen purified from a small cell lung cancer ascitic fluid with affinities of 2 x 108 and 5 x 107 M-1 to the same extent, respectively. Since the functional antigen-binding domain of the anti-EGFRvIII **antibodies** in camels is much simpler and located only on the heavy chains of proteins, we are currently developing recombinant and smaller versions of the variable domain of these naturally occurring heavy-chain **antibodies** (**VHH**) for use in tumor imaging and cancer therapy. Copyright (C) 2004 S. Karger AG, Basel.

L11 ANSWER 7 OF 7 CAPLUS COPYRIGHT 2007 ACS on STN
2005:520389 Document No. 143:265130 Production of **antibody** against **EGFR** V III and the other EGF receptor family and molecular cloning of heavy chain **antibody** repertoire of Camelus bactrianus and Camelus dromedarius. Omidfar, K.; Rasaee, M. J.; Forouzandeh Moghadam, M.; Taghikhani, M.; Modjtahedi, H.; Sadroddiny, E. (Biochemistry Department, School of Medical Sciences, Tarbiat Modarres University, Tehran, Iran). Majmoa-i Maghalat-i Sevomin Hemayesh Maliy Biotechnology Jomhoriy-i Islame-i Iran, Mashhad, Islamic Republic of Iran, Sept. 9-11, 2003, Volume 3, 42-45. Danishgah-i Ferdowsi Mashhad: Mashhad, Iran. ISBN: 964-386-023-X (English) 2003. CODEN: 69GXPF.

AB The epidermal growth factor receptor (**EGFR**) is a membrane glycoprotein that possesses intrinsic protein tyrosine kinase activity and mediates proliferation and differentiation of cells when activated by its ligand EGF or transforming growth factor α (TGF α). Many cancerous cell have been shown to express mutant form of this receptor. The most common receptor mutant, **EGFR** V III, is known to be present in glioblastomas, breast and ovarian cancers, non-small cell lung carcinomas and prostate cancers. This mutated receptor has previously been described and is formed by a 267 amino acid in-frame deletion and an insertion of a glycine in the fusion of the extra cellular domain. Camelidae are known to produce **antibodies** devoid of light chains and CH1 domains. Antigen-specific fragments. of these heavy-chain IgGs (**VHH**) are of great interest in biotechnol. applications. In this study we report the novel polyclonal camel **antibodies** directed to the mutation site of **EGFR** V III. These **antibodies** were generated by immunization of camelids with a synthetic peptide corresponding to the mutated sequence of the receptor, tissue homogenize of several patient with human glioblastoma, medulloblastoma and aggressive breast carcinoma as well as EGF receptor expression cell lines. The elicited **antibody** reacted specifically with the fusion peptide in ELISA. The anti-fusion junction peptide **antibody** selectively bound to the mutated receptor as compared to the intact epidermal growth factor receptor as assessed by immunocytochem. and Western blot anal. In addition to enable the specific and efficient isolation of **VHH** genes from peripheral blood B-cells, the long and short-hinge specific primers were used in the construction of camelids **VHH** libraries. We concluded that, using the technique described, antigen-specific **VHH antibody** fragments are readily accessible from the camelids as well as these **antibodies** are ideal candidates for tumor diagnostic and therapeutic applications.

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=> s (laeremans t?/au or henegouwen p?/au)
L12         111 (LAEREMANS T?/AU OR HENEGOUWEN P?/AU)

=> s l12 and EGFR
L13          15 L12 AND EGFR

=> s l13 and antibod?
L14           9 L13 AND ANTIBOD?

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PROCESSING COMPLETED FOR L14
L15           5 DUP REMOVE L14 (4 DUPLICATES REMOVED)

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L15 ANSWER 1 OF 5 CAPLUS COPYRIGHT 2007 ACS on STN
2007:433802 Document No. 146:440196 Nanobodies specific to **EGFR** and IGF-IR for prophylaxis, diagnosis and treatment of cancer, inflammation, rheumatoid arthritis, psoriasis and hypersecretion of mucus in lung. **Laeremans, Toon**; De Haard, Hans; Hoogenboom, Hendricus Renerus Jacobus Mattheus (Ablynx N.V., Belg.). PCT Int. Appl. WO

2007042289 A2 20070419, 345pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IS, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2006-EP9840 20061011. PRIORITY: US 2005-725939P 20051011.

AB The invention relates to polypeptides and Nanobodies against Epidermal Growth Factor Receptor (**EGFR**) and/or Insulin Growth Factor-I Receptor (IGF-IR). The invention also relates to nucleic acids encoding such Nanobodies and polypeptides; to methods for preparing such Nanobodies and polypeptides; to host cells expressing or capable of expressing such Nanobodies or polypeptides; to compns., and in particular to pharmaceutical compns., that comprise such Nanobodies, polypeptides, nucleic acids and/or host cells; and to uses of such Nanobodies, polypeptides, nucleic acids, host cells and/or compns., in particular for prophylactic, therapeutic or diagnostic purposes.

L15 ANSWER 2 OF 5 MEDLINE on STN DUPLICATE 1

2007098583. PubMed ID: 16738850. Efficient inhibition of **EGFR** signaling and of tumour growth by antagonistic anti-EFGR Nanobodies. Roovers Rob C; **Laeremans Toon**; Huang Lieven; De Taeye Severine; Verkleij Arie J; Revets Hilde; de Haard Hans J; van Bergen en Henegouwen Paul M P. (Department of Molecular Cell Biology, Institute of Biomembranes, Utrecht University, Padualaan 8, CH-3584 Utrecht, The Netherlands.) Cancer immunology, immunotherapy : CII, (2007 Mar) Vol. 56, No. 3, pp. 303-317. Journal code: 8605732. ISSN: 0340-7004. Pub. country: Germany: Germany, Federal Republic of. Language: English.

AB The development of a number of different solid tumours is associated with over-expression of ErbB1, or the epidermal growth factor receptor (**EGFR**), and this over-expression is often correlated with poor prognosis of patients. Therefore, this receptor tyrosine kinase is considered to be an attractive target for **antibody**-based therapy. Indeed, **antibodies** to the **EGFR** have already proven their value for the treatment of several solid tumours, especially in combination with chemotherapeutic treatment regimens. Variable domains of camelid heavy chain-only **antibodies** (called Nanobodies) have superior properties compared with classical **antibodies** in that they are small, very stable, easy to produce in large quantities and easy to re-format into multi-valent or multi-specific proteins. Furthermore, they can specifically be selected for a desired function by phage **antibody** display. In this report, we describe the successful selection and the characterisation of antagonistic anti-**EGFR** Nanobodies. By using a functional selection strategy, Nanobodies that specifically competed for EGF binding to the **EGFR** were isolated from "immune" phage Nanobody repertoires. The selected **antibody** fragments were found to efficiently inhibit EGF binding to the **EGFR** without acting as receptor agonists themselves. In addition, they blocked EGF-mediated signalling and EGF-induced cell proliferation. In an in vivo murine xenograft model, the Nanobodies were effective in delaying the outgrowth of A431-derived solid tumours. This is the first report describing the successful use of untagged Nanobodies for the in vivo treatment of solid tumours. The results show that functional phage **antibody** selection, coupled to the rational design of Nanobodies, permits the rapid development of novel anti-cancer **antibody** -based therapeutics.

L15 ANSWER 3 OF 5 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

2006:547709 The Genuine Article (R) Number: 048RY. Molecular biology of epidermal growth factor receptor inhibition for cancer therapy. Oliveira S (Reprint); **Henegouwen P M V E**; Storm G; Schiffelers R M. Univ

Utrecht, Dept Pharmaceut, Utrecht Inst Pharmaceut Sci, POB 80-082, NL-3508 TB Utrecht, Netherlands (Reprint); Univ Utrecht, Dept Pharmaceut, Utrecht Inst Pharmaceut Sci, NL-3508 TB Utrecht, Netherlands; Univ Utrecht, Inst Biomembranes Mol Cell Biol, NL-3508 TB Utrecht, Netherlands. S.Oliveira@pharm.uu.nl; P.M.P.vanBergenenHenegouwen@bio.uu.nl; G.Storm@pharm.uu.nl; R.M.Schiffelers@pharm.uu.nl. EXPERT OPINION ON BIOLOGICAL THERAPY (JUN 2006) Vol. 6, No. 6, pp. 605-617. ISSN: 1471-2598. Publisher: ASHLEY PUBLICATIONS LTD, TELEPHONE HOUSE, 69-77 PAUL STREET, LONDON EC2A 4LQ, ENGLAND. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Understanding the role of the epidermal growth factor receptor (**EGFR**) in cellular signalling processes underlying malignancy has enabled the development of rationally designed **EGFR**-targeted therapeutics. Strategies have been devised to interfere with the **EGFR** signalling at three different levels: at the extracellular level, competing with ligand binding; at the intracellular level, inhibiting the activation of the tyrosine kinase; or at the mRNA level, modulating the expression of the **EGFR** protein. Each of these strategies has proven to have an antitumour effect mediated by events such as inhibition of cell proliferation, induction of apoptosis, decrease of cellular invasion and migration; and/or inhibition of angiogenesis. Furthermore, the combination of these strategies with traditional chemotherapy or radiotherapy has generally resulted in enhanced antitumour effects. Likewise, the benefit of interfering simultaneously with different signalling pathways has been documented to improve tumour growth inhibition. These preclinical results have encouraged clinical studies that led to the FDA approval of three drugs. However, finding the perfect strategy for each individual patient appears to be a limiting factor, demanding further research to be able to generate relevant molecular expression profiles on a case-to-case basis. Taken together, a successful **EGFR** inhibition will require a better understanding of signalling pathways in combination with the development of rationally designed effective molecules.

L15 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2007 ACS on STN

2005:429443 Document No. 142:480780 Camelid heavy chain **antibodies** directed against epidermal growth factor receptor. **Laeremans, Toon** (Ablynx N. V., Belg.). PCT Int. Appl. WO 2005044858 A1 20050519, 78 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-BE189 20031107.

AB The author discloses the preparation, selection, and characterization of llama **antibodies** directed to human epidermal growth factor receptor.

L15 ANSWER 5 OF 5 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

1992:617613 The Genuine Article (R) Number: JT981. THE EGF RECEPTOR IS AN ACTIN-BINDING PROTEIN. DENHARTIGH J C (Reprint); **HENEGOUWEN P M P V**; VERKLEIJ A J; BOONSTRA J. UNIV UTRECHT, DEPT MOLEC CELL BIOL, 3584 CH UTRECHT, NETHERLANDS (Reprint). JOURNAL OF CELL BIOLOGY (OCT 1992) Vol. 119, No. 2, pp. 349-355. ISSN: 0021-9525. Publisher: ROCKEFELLER UNIV PRESS, 1114 FIRST AVE, 4TH FL, NEW YORK, NY 10021. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB In a number of recent studies it has been shown that in vivo part of the EGF receptor (**EGFR**) population is associated to the actin filament system. In this paper we demonstrate that the purified **EGFR** can be cosedimented with purified filamentous actin (F-actin) indicating a direct association between **EGFR** and actin. A truncated **EGFR**, previously shown not to be associated to the

cytoskeleton, was used as a control and this receptor did not cosediment with actin filaments. Determination of the actin-binding domain of the **EGFR** was done by measuring competition of either a polyclonal **antibody** or synthetic peptides on **EGFR** cosedimentation with F-actin. A synthetic peptide was made homologous to amino acid residues 984-996 (HL-33) of the **EGFR** which shows high homology with the actin-binding domain of Acanthamoeba profilin. A polyclonal **antibody** raised against HL-33 was found to prevent cosedimentation of **EGFR** with F-actin. This peptide HL-33 was shown to bind directly to actin in contrast with a synthetic peptide homologous to residues 1001-1013 (HL-34). During cosedimentation, HL-33 competed for actin binding of the **EGFR** and HL-34 did not, indicating that the **EGFR** contains one actin-binding site. These results demonstrate that the **EGFR** is an actin-binding protein which binds to actin via a domain containing amino acids residues 984-996.

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=> d his

     (FILE 'HOME' ENTERED AT 15:51:50 ON 28 DEC 2007)

     FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 15:52:29 ON
     28 DEC 2007
L1           636 S SINGLE DOMAIN ANTIBOD?
L2            10 S L1 AND EGFR
L3             2 DUP REMOVE L2 (8 DUPLICATES REMOVED)
L4            84 S VHH ANTIBODY
L5            11 S L4 AND EGFR
L6             3 DUP REMOVE L5 (8 DUPLICATES REMOVED)
L7       3060346 S ANTIBOD?
L8          9505 S L7 AND EGFR
L9             0 S L8 AND CAMELIADAE
L10           16 S L8 AND VHH
L11            7 DUP REMOVE L10 (9 DUPLICATES REMOVED)
L12          111 S (LAEREMANS T?/AU OR HENEGOUWEN P?/AU)
L13           15 S L12 AND EGFR
L14            9 S L13 AND ANTIBOD?
L15            5 DUP REMOVE L14 (4 DUPLICATES REMOVED)

=> s l8 and camel
L16           16 L8 AND CAMEL

=> dup remove l16
PROCESSING COMPLETED FOR L16
L17            4 DUP REMOVE L16 (12 DUPLICATES REMOVED)

=> d l17 1-4 cbib abs

L17  ANSWER 1 OF 4     MEDLINE on STN                    DUPLICATE 1
2006718898.    PubMed ID: 16848761.   Studies of thermostability in Camelus
     bactrianus (Bactrian camel) single-domain antibody
     specific for the mutant epidermal-growth-factor receptor expressed by
     Pichia. Omidfar Kobra; Rasaee Mohhamad Javad; Kashanian Soheila; Paknejad
     Malieheh; Bathaie Zahra. (Endocrinology and Metabolism Research Centre,
     Tehran University of Medical Sciences, Tehran, Iran. ) Biotechnology and
     applied biochemistry, (2007 Jan) Vol. 46, No. Pt 1, pp. 41-9.  Journal
     code: 8609465. E-ISSN: 1470-8744. Pub. country: England: United Kingdom.
     Language: English.
AB   Camelids have a unique immune system capable of producing heavy-chain
     antibodies lacking the light chains and CH1 (constant heavy-chain
     domain 1).  It has been shown that, in contrast with conventional
     antibody fragments, the variable domains of these heavy-chain
     antibodies are functional at or after exposure to high
     temperatures.  In the present study, the VHH (variable domain of
     heavy-chain antibody) camel antibody was
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subcloned into vector Ppiczc and expressed in Pichia pastoris. ORB1-83 VHH **antibody** recognizes the external domain of the mutant **EGFR** [EGF (epidermal growth factor) receptor], **EGFR** VIII. This tumour-specific antigen is ligand-independent, contains a constitutively active tyrosine kinase domain and has been shown to be present in a number of human malignancies. We report here that, although expression from P. pastoris resulted in a significantly increased level of expression of the anti-**EGFR** VIII VHH **antibodies** compared with Escherichia coli [Omidfar, Rasaee, Modjtahedi, Forouzandeh, Taghikhani, Bakhtiari, Paknejad and Kashanian (2004) Tumor Biol. 25, 179-187; Omidfar, Rasaee, Modjtahedi, Forouzandeh, Taghikhani and Golmakany (2004) Tumor Biol. 25, 296-305], this **antibody** selectively bound to the **EGFR** VIII peptide and reacted specifically with the immunoaffinity-purified antigen from non-small-cell lung cancer. Furthermore, thermal denaturation stability and CD spectra analysis of the Camelus bactrianus (Bactrian **camel**) VHH and heavy-chain **antibodies** at different temperature proved reversibility and binding activity after heat denaturation. Our results indicate that the P. pastoris expression system may be useful for the expression of **camel** single domain **antibody** and the ability of the expressed protein to reversibly melt without aggregation, allowing it to regain binding activity after heat denaturation.

L17 ANSWER 2 OF 4 MEDLINE on STN DUPLICATE 2

2005002331. PubMed ID: 15627895. Production of a novel **camel** single-domain **antibody** specific for the type III mutant **EGFR.** Omidfar K; Rasaee M J; Modjtahedi H; Forouzandeh M; Taghikhani M; Golmakani N. (Department of Biochemistry, School of Medical Sciences, Tarbiat Modarres University, Tehran, Iran.) Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine, (2004 Sep-Dec) Vol. 25, No. 5-6, pp. 296-305. Journal code: 8409922. ISSN: 1010-4283. Pub. country: Switzerland. Language: English.

AB Camelids have a unique immune system capable of producing single-domain heavy-chain **antibodies.** The antigen-specific domain of these heavy-chain IgGs (VHH) are the smallest binding units produced by the immune system. In this study, we report the isolation and characterization of several binders against the epidermal growth factor receptor (**EGFR**) vIII retrieved from immune library of **camels** (Camelus bactrianus and Camelus dromedarius). The EGFRvIII is a ligand-independent, constitutively active, mutated form of the wild-type **EGFR.** The expression of EGFRvIII has been demonstrated in a wide range of human malignancies, including gliomas, and breast, prostate, ovarian and lung cancer. **Camels** were immunized with a synthetic peptide corresponding to a mutated sequence and tissue homogenates. Single-domain **antibodies** (VHH) were directly selected by panning a phage display library on successively decreasing amounts of synthetic peptide immobilized on magnetic beads. The anti-EGFRvIII **camel** single-domain **antibodies** selectively bound to the EGFRvIII peptide and reacted specifically with the immunoaffinity-purified antigen from a non-small cell lung cancer patient. These **antibodies** with affinities in the nanomolar range recognized the EGFRvIII peptide and affinity-purified mutated receptor. We concluded that using the phage display technique, antigen-specific VHH **antibody** fragments are readily accessible from the camelids. These **antibodies** may be good candidates for tumor-diagnostic and therapeutic applications.

L17 ANSWER 3 OF 4 MEDLINE on STN DUPLICATE 3

2004586121. PubMed ID: 15557755. Production and characterization of a new **antibody** specific for the mutant EGF receptor, EGFRvIII, in Camelus bactrianus. Omidfar K; Rasaee M J; Modjtahedi H; Forouzandeh M; Taghikhani M; Bakhtiari A; Paknejad M; Kashanian S. (Department of Biochemistry, School of Medical Sciences, Tarbiat Modarres University, Tehran, I.R. Iran.) Tumour biology : the journal of the International

Society for Oncodevelopmental Biology and Medicine, (2004 Jul-Aug) Vol. 25, No. 4, pp. 179-87. Journal code: 8409922. ISSN: 1010-4283. Pub. country: Switzerland. Language: English.

AB EGFRvIII is the type III deletion mutant form of the epidermal growth factor receptor (**EGFR**) with transforming activity. This tumor-specific antigen is ligand independent, contains a constitutively active tyrosine kinase domain and has been shown to be present in a number of human malignancies. In this study, we report the production and characterization of **camel antibodies** that are directed against the external domain of the EGFRvIII. **Antibodies** developed in **camels** are smaller (i.e. IgG2 and IgG3 subclasses lack light chains) than any other conventional mammalian **antibodies**. This property of **camel antibodies** makes them ideal tools for basic research and other applications such as tumor imaging and cancer therapy. In the present study, **camel antibodies** were generated by immunization of camelids (Camelus bactrianus and Camelus dromedarius) with a synthetic 14-amino acid peptide corresponding to the mutated sequence of the **EGFR**, tissue homogenates of several patients with human glioblastoma, medulloblastoma and aggressive breast carcinoma, as well as **EGFR**-expressing cell lines. Three subclasses of **camel** IgG [conventional (IgG1, 160 kD) and heavy chain-only **antibodies** (IgG2 and IgG3, 90 kD)] were separated by their different binding properties to protein A and protein G affinity columns. The anti-EGFRvIII peptide **antibodies** from immunized **camels** were purified further using the EGFRvIII synthetic peptide affinity column. The purified anti-EGFRvIII peptide **camel antibodies** selectively bound to the EGFRvIII peptide and affinity-purified EGFRvIII from malignant tissues and detected a protein band of 140 kD from malignant tissues by Western blot. Affinity analysis showed that the **antibodies** from C. bactrianus and C. dromedarius reacted with peptide and antigen purified from a small cell lung cancer ascitic fluid with affinities of 2 x 10(8) and 5 x 10(7)M(-1) to the same extent, respectively. Since the functional antigen-binding domain of the anti-EGFRvIII **antibodies** in **camels** is much simpler and located only on the heavy chains of proteins, we are currently developing recombinant and smaller versions of the variable domain of these naturally occurring heavy-chain **antibodies** (V(HH)) for use in tumor imaging and cancer therapy.

L17 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2007 ACS on STN

2005:520389 Document No. 143:265130 Production of **antibody** against **EGFR** V III and the other EGF receptor family and molecular cloning of heavy chain **antibody** repertoire of Camelus bactrianus and Camelus dromedarius. Omidfar, K.; Rasaee, M. J.; Forouzandeh Moghadam, M.; Taghikhani, M.; Modjtahedi, H.; Sadroddiny, E. (Biochemistry Department, School of Medical Sciences, Tarbiat Modarres University, Tehran, Iran). Majmoa-i Maghalat-i Sevomin Hemayesh Maliy Biotechnology Jomhoriy-i Islame-i Iran, Mashhad, Islamic Republic of Iran, Sept. 9-11, 2003, Volume 3, 42-45. Danishgah-i Ferdowsi Mashhad: Mashhad, Iran. ISBN: 964-386-023-X (English) 2003. CODEN: 69GXPF.

AB The epidermal growth factor receptor (**EGFR**) is a membrane glycoprotein that possesses intrinsic protein tyrosine kinase activity and mediates proliferation and differentiation of cells when activated by its ligand EGF or transforming growth factor α (TGF α). Many cancerous cell have been shown to express mutant form of this receptor. The most common receptor mutant, **EGFR** V III, is known to be present in glioblastomas, breast and ovarian cancers, non-small cell lung carcinomas and prostate cancers. This mutated receptor has previously been described and is formed by a 267 amino acid in-frame deletion and an insertion of a glycine in the fusion of the extra cellular domain. Camelidae are known to produce **antibodies** devoid of light chains and CH1 domains. Antigen-specific fragments. of these heavy-chain IgGs (VHH) are of great interest in biotechnol. applications. In this study we report the novel polyclonal **camel antibodies** directed to the mutation site of **EGFR** V III. These **antibodies**

were generated by immunization of camelids with a synthetic peptide corresponding to the mutated sequence of the receptor, tissue homogenize of several patient with human glioblastoma, medulloblastoma and aggressive breast carcinoma as well as EGF receptor expression cell lines. The elicited **antibody** reacted specifically with the fusion peptide in ELISA. The anti-fusion junction peptide **antibody** selectively bound to the mutated receptor as compared to the intact epidermal growth factor receptor as assessed by immunocytochem. and Western blot anal. In addition to enable the specific and efficient isolation of VHH genes from peripheral blood B-cells, the long and short-hinge specific primers were used in the construction of camelids VHH libraries. We concluded that, using the technique described, antigen-specific VHH **antibody** fragments are readily accessible from the camelids as well as these **antibodies** are ideal candidates for tumor diagnostic and therapeutic applications.

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---Logging off of STN---

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Executing the logoff script...

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COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	84.44	84.65

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-6.24	-6.24

STN INTERNATIONAL LOGOFF AT 15:58:10 ON 28 DEC 2007